Journal of Functional Foods 18 (2015) 411–422

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Polysaccharides from *Flammulina velutipes* improve scopolamine-induced impairment of learning and memory of rats

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ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form 21 July 2015
Accepted 12 August 2015
Available online

Keywords:
Flammulina velutipes
Polysaccharides
Learning and memory
Morris water maze
Neurotransmitter
CaMK II

ABSTRACT

Flammulina velutipes has been reported to be beneficial in learning and memory capabilities, but the mechanisms underlying this remain unclear. In this study, Morris water maze and biochemical analyses of rat brain were used to evaluate the effects of *F. velutipes* polysaccharides (FVP) on scopolamine-induced learning and memory impairments. Results suggested that FVP significantly decreased the escape latency and total swimming distance of rats in the hidden platform test and increased the numbers of platform crossing and swimming distance of rats in the probe test. Biochemical examinations revealed that FVP significantly elevates SOD and GSH-Px activities, as well as neurotransmitter levels. The increased acetylcholine content owed to the increased acetylcholine acetyltransferase activity and decreased acetylcholinesterase activity. Moreover, learning and memory associated signalling pathways were activated by FVP elevating the expression of connexin 36 and p-CaMK II. These results conclusively proved that FVP is a potent agent against the progression of cognitive impairment.

1. Introduction

The worldwide population ageing has increased the incidence of cognitive deficits, such as the age-associated memory impairment senile dementias and Alzheimer's disease (AD). AD, characterized by progressive cognitive decline and memory loss, is the sixth leading cause of all deaths in the United States (Alzheimer's Association, 2014). Dozens of drugs and therapies aimed at suppressing learning and memory impairment are being studied by scientists around the world, but at present, there are no effective approaches that could be applied in inhibiting the progression of AD.

It has been suggested that some fruits and vegetables play an important role in delaying the onset of AD (Loef & Walach, 2012). Therefore, increased research is focusing on rational diet

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http://dx.doi.org/10.1016/j.jff.2015.08.003
1756-4646/

to prevent and/or cure AD. The positive cognition effects of polysaccharides, developed as multifunctional components in natural products, have been reported in human as well as in animal studies, such as promoting the induction of long-term potentiation (Edagawa, Smriga, Nishiyama, & Saito, 2001; Hirano et al., 2003), enhancing hippocampal plasticity and behavioural performance (Lin et al., 2014), and reversing scopolamine impaired spatial memory (Chen et al., 2014). Therefore, pursuing and exploring new polysaccharide resources possessing a high cognition function is significant for preventing incidence of cognitive deficits.

Flammulina velutipes, the fourth most popular edible mushroom worldwide due to its delicious taste and high nutritional properties (Chang, Hsiao, et al., 2013; Jing et al., 2014), was extensively considered beneficial in improving learning and memory capabilities. In our previous study, polysaccharide from *F. velutipes* (FVP) was obtained and proved a potential inhibitor of acetylcholinesterase (AChE) activity *in vitro* (Yang, Fang, Liang, & Hu, 2011), which is considered as an effective way to improve cognitive deficit and learning and memory impairment (Mans, Warmus, Smith, & McMahon, 2014). However, limited information is available to elucidate the mechanism underlying this. Therefore, it is essential and desirable to further research the improvement of cognitive deficit by FVP.

Hippocampus and cerebral cortex are major regions of human and other mammal brains. They play important roles in the acquisition, modification and consolidation of information from short-term memory to long-term memory and spatial navigation (Preston & Eichenbaum, 2013). Some pathological reactions and the changes of some chemical components were identified in hippocampus and cerebral cortex when AD occurred (Kosenko et al., 2014). Acetylcholine (ACh), dopamine (DA), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) are essential neurotransmitters for information transmission in the hippocampus (Zheng, 2009), and their changes directly affect the transduction of learning and memory-associated signals (Lanari, Amenta, Silvestrelli, Tomassoni, & Parnetti, 2006). Activation of some synapse protein kinase molecules by means of phosphorylation are involved in learning and memory processes. Phosphorylation of calmodulin-dependent protein kinase II (CaMK II) was reported to be involved in neurotransmitter biosynthesis and secretion and long-term potentiation (Yang et al., 2010). Cell gap junction protein connexin 36 was known as a channel of nutrients and metabolites transportation, and the expression of connexin 36 plays critical roles in neural signal transduction and information exchange between adjacent neurons (Steffensen et al., 2011). All the above alterations were significantly correlated with learning and memory function. Nevertheless, the mechanism of FVP in improving the learning and memory function is still poorly studied. Accordingly, it is significant to analyse the effect of FVP on pathological changes of hippocampus and cerebral cortex.

The aim of this study was to investigate the effect of FVP on the cognitive performance of rats as assessed by hidden platform test and probe test using Morris water maze (MWM). The levels of neurotransmitters and the activities of neuroregulation enzymes in the hippocampus and the cerebral cortex were also evaluated. Moreover, signal pathway associated with learning and memory was further analysed by measuring the expressions of p-CaMK II and connexin 36.

2. Materials and methods

2.1. Materials and chemicals

Fresh *F. velutipes* was purchased from a local market (Nanjing, China). Commercial kits used for determining the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and choline acetyltransferase (ChAT) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Enzyme-linked immunosorbent assay (ELISA) kits used for the contents of 5-hydroxytryptamine (5-HT), dopamine (DA), and norepinephrine (NE) were from Abnova (Taipei, Taiwan). Commercial kits used for determining acetylcholine (ACh) and thiobarbituric acid reactive species (TBARS) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Activity assay kit of acetylcholinesterase (AChE) and scopolamine were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Anti-connexin 36 rabbit polyclonal antibody and anti-p-CaMK II rabbit polyclonal antibody were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other reagents were of analytical grade.

2.2. Preparation of FVP

Fresh *F. velutipes* was dried at 60 °C, powdered and sieved through a No.300 mesh. According to our previous study (Yang et al., 2011), the polysaccharides were prepared with distilled water using an ultrasonic cell disintegrator (DCTZ-2000, Beijing Hongxianglong Biotechnology Development Co. Ltd., Beijing, China) under an optimized condition (ratio of water to material of 25 mL/g, ultrasonic power of 620 W, ultrasonic time of 20 min, and ultrasonic temperature of 45 °C). Crude polysaccharides extract was further deproteinized with Sevag reagent (chloroform:butanol, 4:1), and then precipitated with 4-fold volume anhydrous ethanol. After centrifugation at 2600 × *g* for 15 min, the precipitate was washed successively with anhydrous ethanol and acetone, dialysed against deionized water, and lyophilized as FVP (Yang et al., 2012). The purity of FVP used in the following experiments reached 93 ± 2%.

2.3. Experimental procedures of animals

One hundred and twenty male Wistar rats (40 days old, 200 ± 10 g) were purchased from the Shanghai Experimental Animal Center of Chinese Academy of Science (certificate No. SCXK (Hu) 2012-0005) and housed individually in standard cages in a controlled environment (21 ± 1 °C) with a 12:12 hour light:dark cycle. All rats received *ad libitum* chow and water. The rats were randomly assigned into five groups and treated as follows (Fig. 1A): groups 1 and 2 were treated with distilled water by intragastric administration and named control group and scopolamine-treated group, respectively. Groups 3~5 received FVP in aqueous solution at concentrations of 100, 200, and 400 μg/kg·bw each day by intragastric administration, respectively. After a 30 continuous-day feeding, all the rats were subjected to Morris water maze (MWM) test.

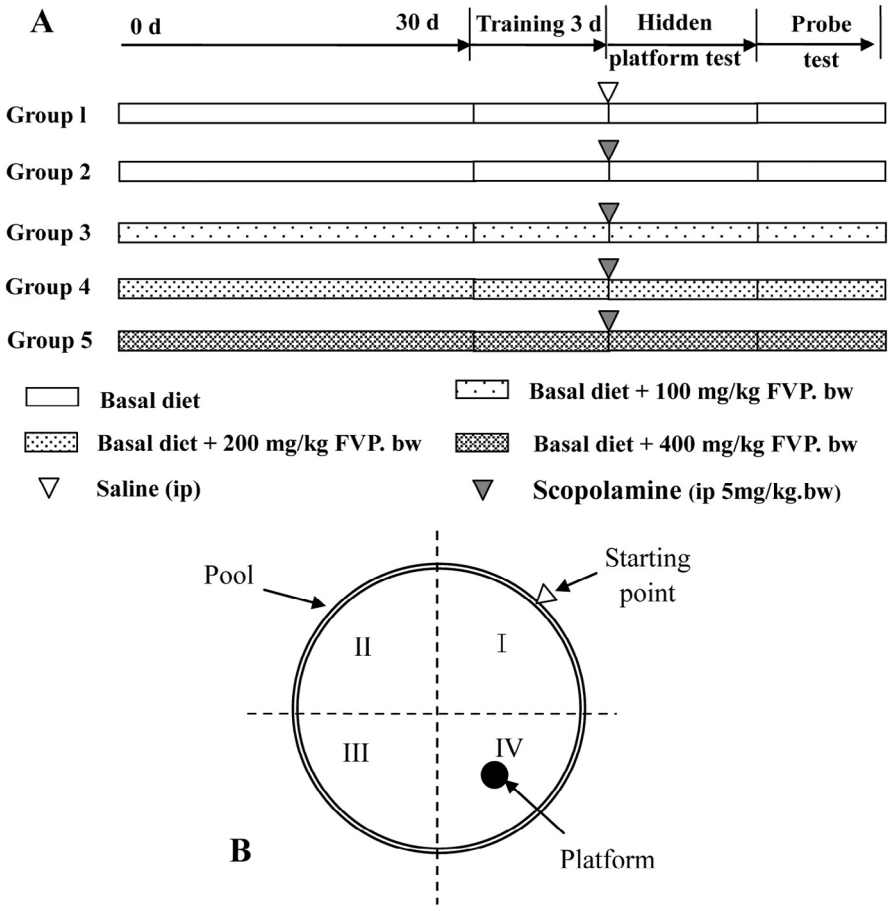


Fig. 1 – Experimental design (A) and range defining of Morris water maze (B) to evaluate the effect of polysaccharides from *Flammulina velutipes* on learning and memory of rats.

After a 30 continuous-day feeding, all rats received three daily cognitive training in MWM which was performed following the method described by Zou et al. (2009). Four quadrants (I, II, III, and IV) of the maze tank (diameter 120 cm, height 45 cm) were designated as shown in Fig. 1B. Starting point was designated in "I" quadrant, the hidden platform (diameter 9 cm, height 20~30 cm) was located in the "IV" quadrant of the tank and was submerged 1 cm below water surface. Each rat was placed in the water facing the wall of the tank at the starting point and the rats were allowed to swim and find the hidden platform location. During each trial, a rat was given 90 s to find the hidden platform. If still in the water after 90 s, the rat was gently guided to the platform and placed on it for 10 s. All testing began at 09:30.

2.4. *Hidden platform test*

After a 3 continuous-day training, the rats of groups 2~5 were injected intraperitoneally (ip) with a dose of 5.0 mg/kg·bw scopolamine to induce cognitive impairment (Yee et al., 2011). Group 2 only treated with scopolamine was named scopolamine-treated group, and groups 3~5 treated with FVP in different dosages and scopolamine were named FVP-treated groups. The rats of group 1 received an intraperitoneal injection with the same amount of saline as the control group. Hidden platform test was performed 10 min later. The time required for the rats to reach the platform (escape latency), the total distance swam for each trial, and the swimming path in the pool were recorded using a video camera connected to a tracking analysis system (DigBehv-MWM, Shanghai Jiliang Software Technology Co. Ltd., Shanghai, China). Hidden platform test was performed for three continuous days.

2.5. *Probe test*

The platform was removed from the pool on the next day after the hidden platform test, and rats were challenged to a single search trial for 120 s (probe test). For the probe test, three parameters were measured, including the number of crossings of the exact place where the platform had been located, the swimming distance in the quadrant of the former platform position, and the swimming path in the pool (Ma et al., 2013).

2.6. *Preparation of brain tissue supernatant*

After probe test, all rats were sacrificed by dislocating their cervical vertebrae. The whole intact brain was quickly removed from the skull and placed on an ice-chilled petri dish for cleaning. We should take care of the meninges as these could rupture the brain and carefully cut the cranial nerves up while taking

out the brain. The cerebral cortex is the cerebrum's (brain) outer layer of neural tissue. Then we gently held the brain in position with the large curved forceps and opened the cortical halves slowly from the midline in a closed position with a small curved forceps. The initial white-coloured part encountered is most likely the corpus callosum, under it is the hippocampus (Li, 2011). Hippocampus and cerebral cortex were rapidly separated, frozen in liquid nitrogen, weighed accurately, and homogenized in 0.95% NaCl (10%, w/v), respectively. The homogenates were centrifuged at 14,000 × *g* for 10 min at 4 °C, and the supernatant were used for following biochemical analyses.

2.7. SOD and GSH-Px activities in hippocampus and cerebral cortex

SOD and GSH-Px activities were investigated with commercially available detection kits according to the manufacturer's instructions. Xanthine/xanthine oxidase system was utilized as the source of superoxide radicals, and SOD activity was regarded as the eliminating superoxide radicals capability (Dong, Wang, Zhang, Yu, & Liu, 2014). The evaluation of GSH-Px activity was based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalysed by GSH-Px (Chang, Lin, et al., 2013). Protein concentration in homogenates was measured by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and all enzyme activities were expressed as units/mg of protein.

2.8. Lipid peroxidation index using thiobarbituric acid reactive species (TBARS)

TBARS was spectrophotometrically determined using a kit based on formation of thiobarbituric acid/malondialdehyde complex (TBA–MDA). TBARS concentrations were determined by measuring absorbance at 532 nm. A calibration curve was constructed with MDA as the standard. The results were expressed as nmol TBARS/mg protein.

2.9. Neurotransmitter contents in hippocampus and cerebral cortex

The levels of 5-HT, DA, and NE in hippocampus and cerebral cortex were measured using ELISA kits according to the manufacturer's instructions. Dispensed antigen standards and samples were added to each well of 96-well plates precoated with primary antibodies. After adding biotin conjugate reagent and enzyme conjugate reagent into each well, the plates were incubated at 37 °C for 30 min. Then the plates were rinsed 5 times with distilled water. Within 30 min of the chromogenic reaction, the absorbance was read at 450 nm using a microplate reader (Thermo Multiskan Mk3, Rockford, IL, USA) (Zhou, Liu, Yan, Cao, & Liu, 2012).

ACh content was determined spectrophotometrically using a commercial kit according to the manufacturer's instruction, which is based on the colorimetric determination of the red purple colour produced by the binding between hydroxamic acid and ferric ion (Kim et al., 2010). ACh chloride was used as a standard. Absorbance was measured at 550 nm with a UV/visible spectrophotometer (Amersham Biosciences, Buckinghamshire, UK).

2.10. AChE and ChAT activities in hippocampus and cerebral cortex

AChE activity was determined with AChE activity assay kit according to the manufacturer's instruction. Briefly, ACh as a substrate was hydrolysed by AChE to acetate and choline, and the latter could react with 5,5′-Dithiobis-(2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid, which absorbs at 412 nm (Phyu & Tangpong, 2014). ChAT activity was determined by the ACh synthesis reaction that ChAT catalysed (Acetyl-CoA + choline → Acetylcholine + Co-enzyme A) and performed according to the manufacturer's instruction (Lu et al., 2014). 0.95% NaCl was used as a blank control in the determination of AChE and ChAT activities. The activities of AChE and ChAT were expressed in U/mg protein.

2.11. Western blot analysis

Western blotting analysis was carried out as described previously with minor modifications (Yamamoto et al., 2009; Zou et al., 2009). Hippocampus and cerebral cortex were homogenized in buffer containing 50 mM Tris–HCl (pH 7.4), 0.5%Triton X-100, 4 mM ethyleneglycoltetraacetic acid (EGTA), 10 mM ethylenediaminetetraacetic acid (EDTA), 1 mM Na_3VO_4, 40 mM sodium pyrophosphate, 50 mM NaF, 100 nM calyculin A, 50 μg/mL leupeptin, 25 μg/mL pepstatin A, 50 μg/mL trypsin inhibitor and 1 mM dithiothreitol. The homogenates were centrifuged at 4 °C and total protein was measured by a Bradford protein assay kit using bovine serum albumin (BSA) as the standard. Proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (5% SDS-PAGE) and then transferred to nitrocellulose membranes. After blocking with TBST solution (50 mM Tris–HCl, pH 7.5, 150 mM NaCl and 0.1% Tween 20) containing 5% skim milk for 1 h at room temperature, the membranes were incubated overnight at 4 °C with anti-β-actin antibody (1:1000), anti-p-CaMK II antibody (1:1000), and anti-connexin 36 antibody (1:400). Bound antibodies were visualized using enhanced chemiluminescence system (Amersham Bio-sciences) and analysed semiquantitatively using the quantity one v4.62 software.

2.12. Statistical analysis

All data from 24 replicated determinations (n = 24) were analysed and reported as means ± standard deviations (SDs). SPSS 12.0 software was used for analysis of variance (ANOVA), and the differences among means were determined by the least significance difference test with significance defined at $P < 0.05$.

3. Result and discussion

3.1. Behavioural performance of rats in hidden platform test

Hidden platform test and probe test of MWM are widely used to assess the spatial learning and memory capability of rats

in laboratory (Morris, 1984). In general, Swimming paths of rats in MWM test can be distinguished into four types of search strategies: (1) marginal: swimming along the pool edge; (2) random: swimming randomly and searching platform without purpose; (3) taxis: swimming around but towards the platform area, which was regarded to be relative to the abilities of rats to acquire, consolidate, and retain spatial information; (4) straight: swimming straight towards the platform (Wang & Han, 2009). The straight and taxis strategies are more efficient ways of finding the platform, while marginal and random strategies are inferior ways that are less efficient for escaping from drowning. Meanwhile, escape latency and swimming path distance were also analysed for the evaluation of rats' spatial learning capabilities. The escape latency spent to reach the platform indicates how quickly it can learn and memorize the location of the platform, and shorter escape latency as compared to the untreated control indicates an improvement in spatial learning and memory.

The representative swimming paths, escape latency and swimming path distance of rats in hidden platform test were shown in Fig. 2A–C. After a 3-day training, all groups of rats could quickly find the location of the platform using more taxis and straight search strategies. Scopolamine was used extensively to induce learning and memory deficit of animals. After scopolamine treatment, the search strategies of rats were of the characteristics of randomness and irregularity, and the rats lost use of taxis and straight search strategies and relied more on marginal or random search strategies (Fig. 2A, scopolamine-treated group), which was corresponding to the significantly longer swimming path distance and escape latency compared with untreated group 1 ($p < 0.05$). The swimming path distance ranged from 539 cm of control group to 2511 cm of the scopolamine-treated group, and escape latency ranged from 28 s of the control group to 89 s of the scopolamine-treated group. The deterioration performance of scopolamine-treated group implied the markedly neurological impairment in rats induced by scopolamine.

FVP-administered rats treated with scopolamine (group 3~5) performed significantly better spatial search behaviour than scopolamine-treated group (group 2), and their predominant search strategy was taxis style, which indicated that learning and memory impairment were attenuated by administration of FVP. That was mainly explained by the significantly shorter escape latency and swimming path distance in the area where the platform was placed. The effect of FVP on reversing scopolamine-induced brain issue impairment gradually increased with the increase of polysaccharide dose. Compared with scopolamine-treated group, the escape latency of rats treated with 400 μg/kg FVP significantly reduced and was not significantly different with that of control group ($p > 0.05$).

3.2. *Behavioural performance of rats in probe test*

The search strategies as well as numbers and swimming distance of rats across the platform was analysed in the probe test to evaluate the spatial memory capability of rats. Representative swimming paths of rats in the probe test were shown in Fig. 2D–F. Swimming search strategies of control group rats were mainly taxis and straight, whereas the search strategies of scopolamine-treated group were mainly marginal and random. The numbers and swimming distance of rats crossing the platform location decreased from 2.6 times and 328 cm of control group to 1.7 times and 189 cm of scopolamine-treated group, respectively. Spatial search behaviour of group 3~5 was significantly better in probe test than that of scopolamine-treated group (group 2), and the effect of FVP on preventing the deterioration of behavioural performance was in a concentration dependent manner. These results indicated that FVP is effective in delaying the scopolamine-induced memory impairment, which confirmed that there is potential for *F. velutipes* to improve the learning and memory of animals.

3.3. *SOD and GSH-Px activities and MDA levels in hippocampus and cerebral cortex*

Oxidative stress contributes to the progression of AD by inducing brain tissue impairment and learning and memory deficits (Pohanka, 2013). SOD and GSH-Px were considered to be two important antioxidant enzymes to drive off oxidative stress. Some studies have shown that memory impairment was associated with increased lipid peroxidation levels and reduced SOD and GSH-Px activities in brain (Gao et al., 2012). Therefore, SOD and GSH-Px activities in hippocampus and cerebral cortex were further investigated. The results suggested that SOD and GSH-Px activities of scopolamine-treated group rats were significantly decreased compared with that of the control group (Fig. 3), which suggested that scopolamine could elevate oxidative damage in brain characterized by inactivation of SOD and GSH-Px. Thirty-day administration of FVP prevented the decrease of SOD and GSH-Px activities induced by scopolamine. TBARS is a marker of membrane lipid peroxidation which can be induced by oxidative stress. Therefore, TBARS concentration was used to denote the oxidative stress. We found that scopolamine treatment markedly elevated the TBARS concentrations in the hippocampus and the cerebral cortex, which was significantly attenuated by the FVP pre-treatment in a dose-dependent manner. These indicated that FVP could effectively improve the memory deficits in association with ameliorating oxidative stress. Some studies have revealed that acidic oligosaccharide sugar chain could induce cognitive improvement via its antioxidant activity (Fan et al., 2005), and a sulphated polysaccharide extracted from brown algae enhanced the activities of SOD and GSH-Px in hippocampus, and reduced lipid peroxidation (Gao et al., 2012), which is consistent with our conclusion. These findings confirmed that FVP ameliorated the learning and memory capabilities by elevating antioxidant enzymes activities and relieving oxidative stress.

3.4. *Neurotransmitter contents in hippocampus and cerebral cortex*

Evidence revealed that cholinergic mechanisms modulate learning and memory formation. Some neurotransmitters such as ACh, DA, 5-HT and NE were thought to play an important role in learning and memory, and the levels of neurotransmitters in the hippocampus and cerebral cortex altered with the occurrence of evident severe cognitive deficits (Wang et al., 2013).

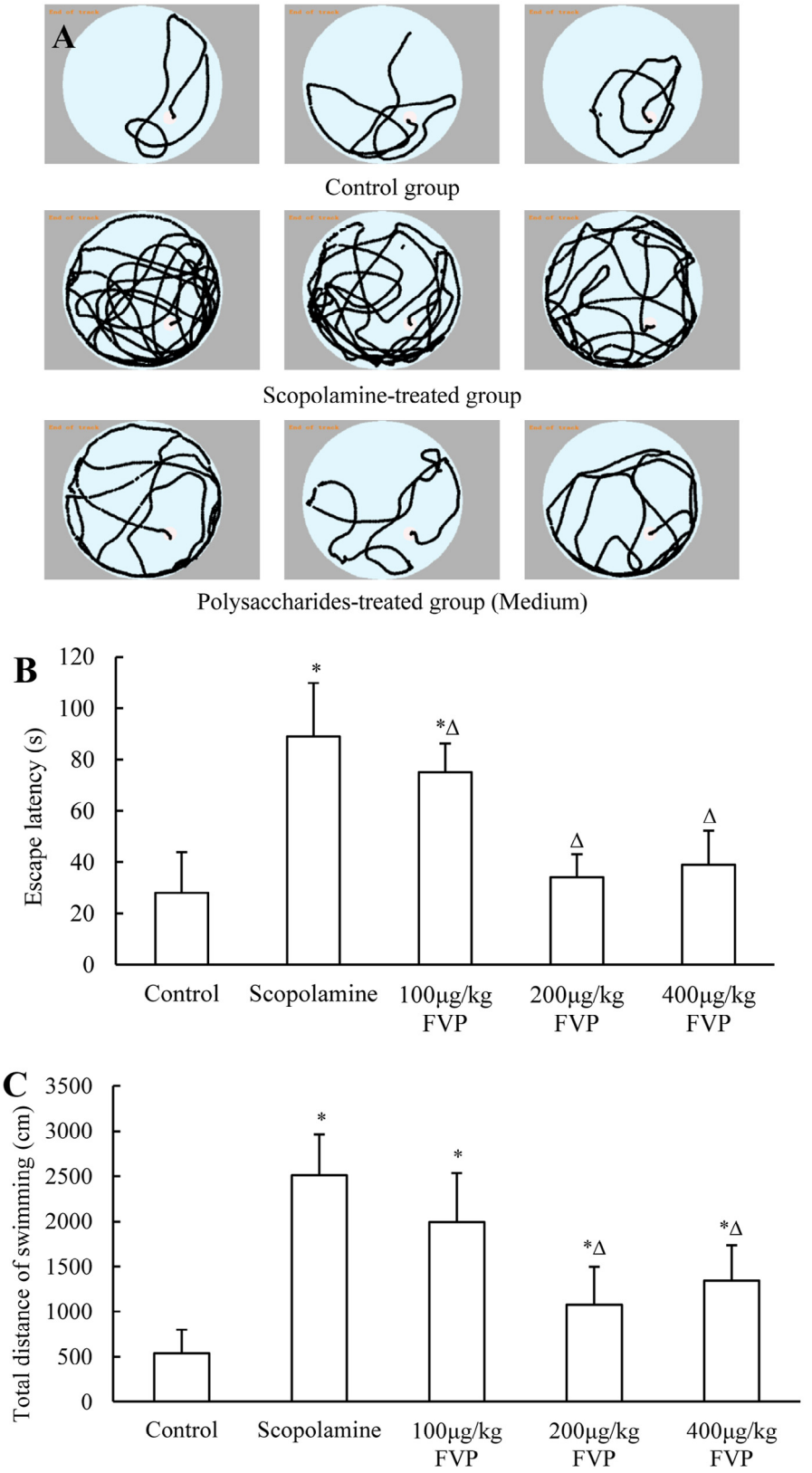
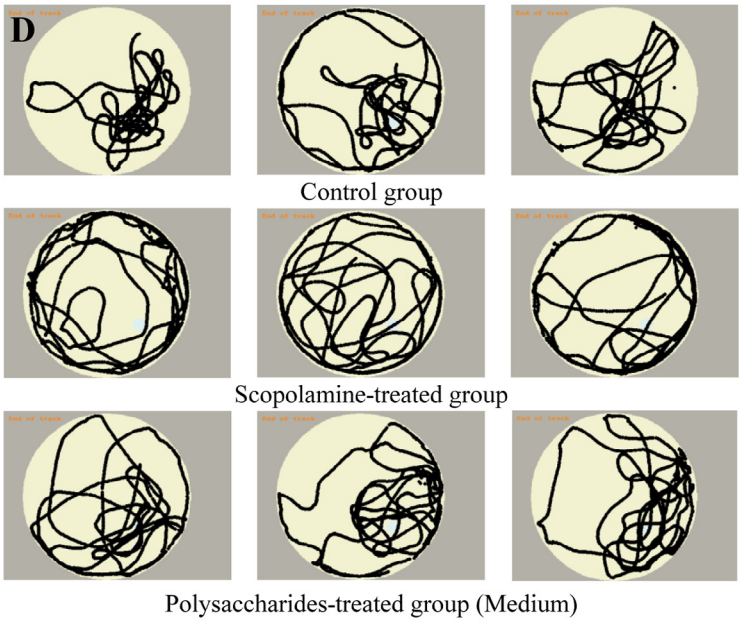
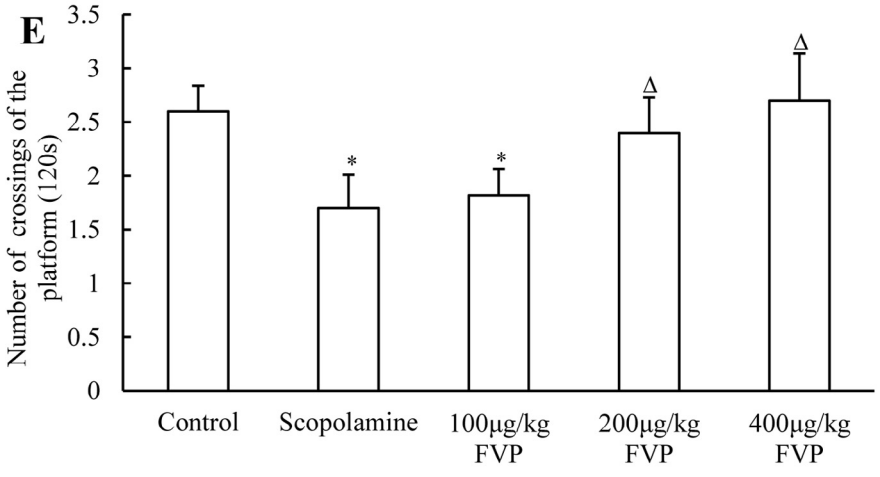


Fig. 2 – Representative swimming paths (A), escape latency (B) and total swimming distance (C) of rats in hidden platform test of the Morris water maze. Representative swimming paths (D), number of crossings of the platform (E) and distance of moving around the platform (F) of rats in probe test of Morris water maze. $^{*}P < 0.05$ vs control and $^{\Delta}P < 0.05$ vs scopolamine.





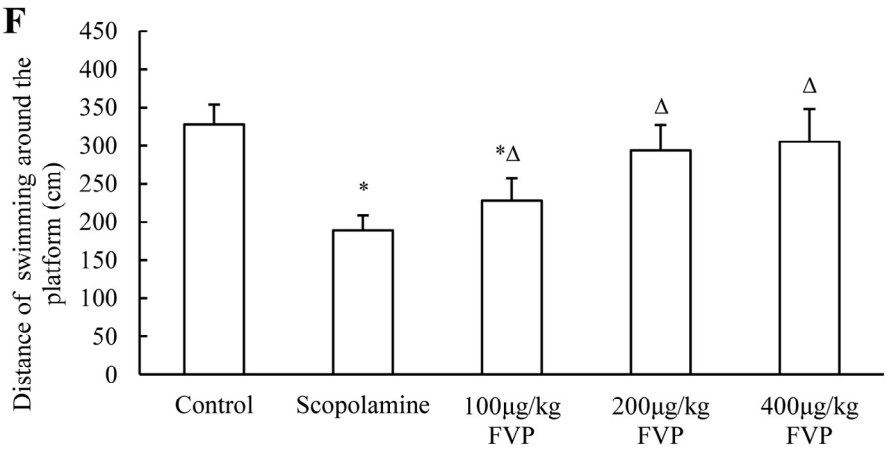


Fig. 2 – (*continued*)

In order to elucidate the mechanism of FVP in improving learning and memory ability, the changes of neurotransmitters in the hippocampus and cerebral cortex were further investigated.

Results showed that scopolamine treatment significantly decreased the contents of ACh, 5-HT, DA and NE in the hippocampus and the cerebral cortex (Fig. 4). Compared with scopolamine-treated group, FVP reversed the

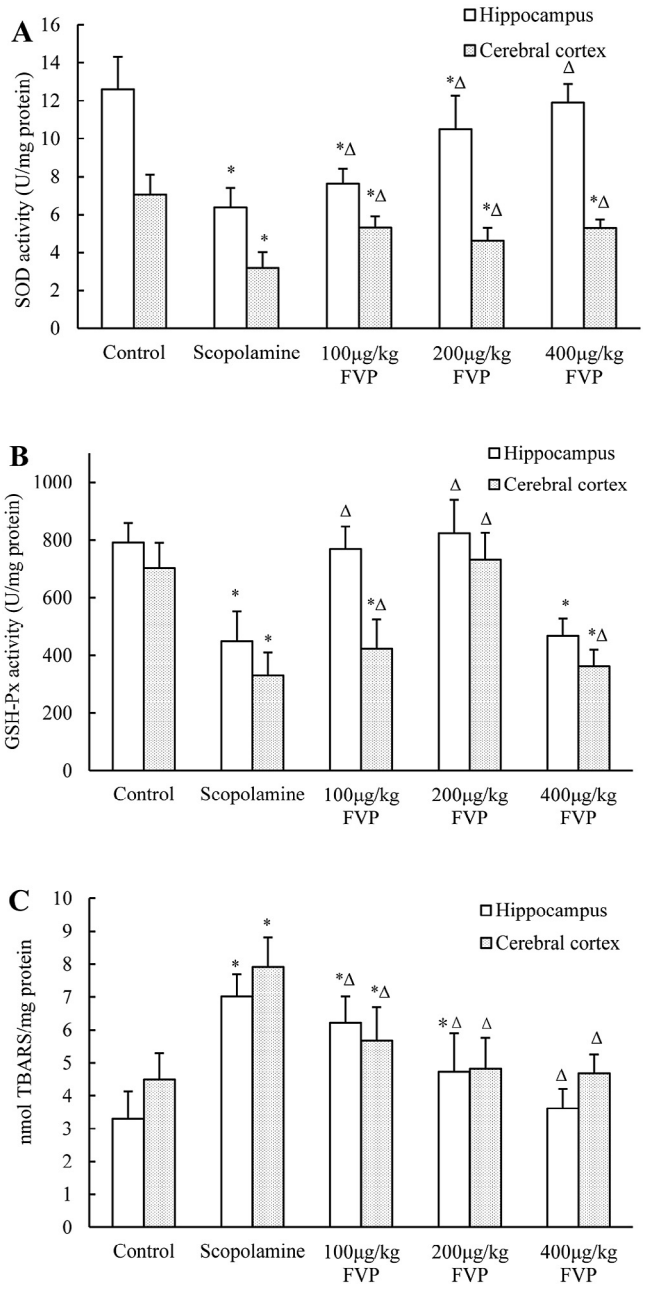


Fig. 3 – The SOD (A) and GSH-Px (B) activities and TBARS concentrations (C) in hippocampus and cerebral cortex of rats in different groups. *P < 0.05 vs control and ΔP < 0.05 vs scopolamine.

scopolamine-induced decrease of ACh, 5-HT, DA, and NE levels, especially the effect of FVP at a high dose on ACh, DA, and NE levels in the hippocampus. Some studies have shown that the neurotransmitter dysfunction and decreases in ACh and DA levels have resulted in patients with learning and memory injury. Furthermore, DA deficits are important in AD because it has been shown to play an important role in attention, anxiety and depression (Geerts, Lazarewicz, Spiros, Carr, & Finkel, 2003). FVP regulating the levels of ACh, DA, 5-HT and NE was thought to be an effective way to restrain the learning and memory impairment and progression of AD.

3.5. *ChAT and AChE activities in the hippocampus and the cerebral cortex*

The dysregulation of ACh is an important symptom of learning and memory impairment. We have proven that FVP could restore the level of ACh in the hippocampus and the cerebral cortex, but the molecular mechanism underlying this remains unclear. ChAT is a critical enzyme for ACh synthesis in the cytoplasm of autonomic nerve terminals. Less active ChAT in AD brain than that in a non-Alzheimer's brain resulted in a reduction of ACh synthesis (Hut & Van der Zee, 2011). AChE, a key enzyme for cholinergic transmission in the nervous system, is remarkably efficient in hydrolysing ACh into acetate and choline and functions at cholinergic synapses by terminating the chemical impulse of ACh (Silman & Sussman, 2005). Therefore, ChAT and AChE activities in the hippocampus and the cerebral cortex were further investigated to clarify the mechanism of the ACh dysregulation. Results showed that scopolamine significantly decreased ChAT activity and increased AChE activity in the hippocampus and the cerebral cortex (Fig. 5), which was reversed by FVP administration. These results indicated that FVP restored the level of ACh by modulating the ChAT and AChE activities. This conclusion was supported by the evidence that polysaccharides from *Millettia pulchra*, *brown algae* and *Schisandra chinensis* regulated the accumulation and metabolism of ACh by inhibiting AChE activity (Lin et al., 2014) and/or elevating ChAT activity (Gao et al., 2012; Miao, Gao, Zhang, Ma, & Zhang, 2009).

3.6. *Expression of connexin 36 and p-CaMK II in hippocampus and cerebral cortex*

It has been reported that the phosphorylation and activation of some protein kinases such as CaMKII and connexin 36 may be involved in the biosynthesis and secretion of neurotransmitters. CaMK II is highly expressed in neuronal tissues and regulates the biosynthesis and exocytosis of neurotransmitters. Autophosphorylation has proven to be a characteristic property of CaMK II activation, which was relative to the formation of spatial learning and memory and essential for long-term potentiation in the hippocampus (Lisman, Yasuda, & Raghavachari, 2012). CaMK II also increased the conductance at gap junctions which were formed by the neuronal gap junction protein connexin 36. Therefore, neurochemical analysis was further performed to clarify whether neurotransmitter changes were associated with FVP regulating the expression of some protein. Results suggested that, compared with the control group, scopolamine treatment significantly reduced the expression levels of p-CaMK II and connexin 36 in hippocampus and cerebral cortex, which was apparently inhibited by FVP in a concentration dependent manner (Fig. 6). Statistically significant differences of p-CaMK II and connexin 36 expression were not observed between group 5 (treated with high dose of FVP) and the control group. These results indicated that FVP ameliorates learning and memory impairments by regulating the expression of CaMK II and connexin 36, which is probably the main pathway of FVP in improving scopolamine-induced impairment of learning and memory of rats.

It is reported that the blood–brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood

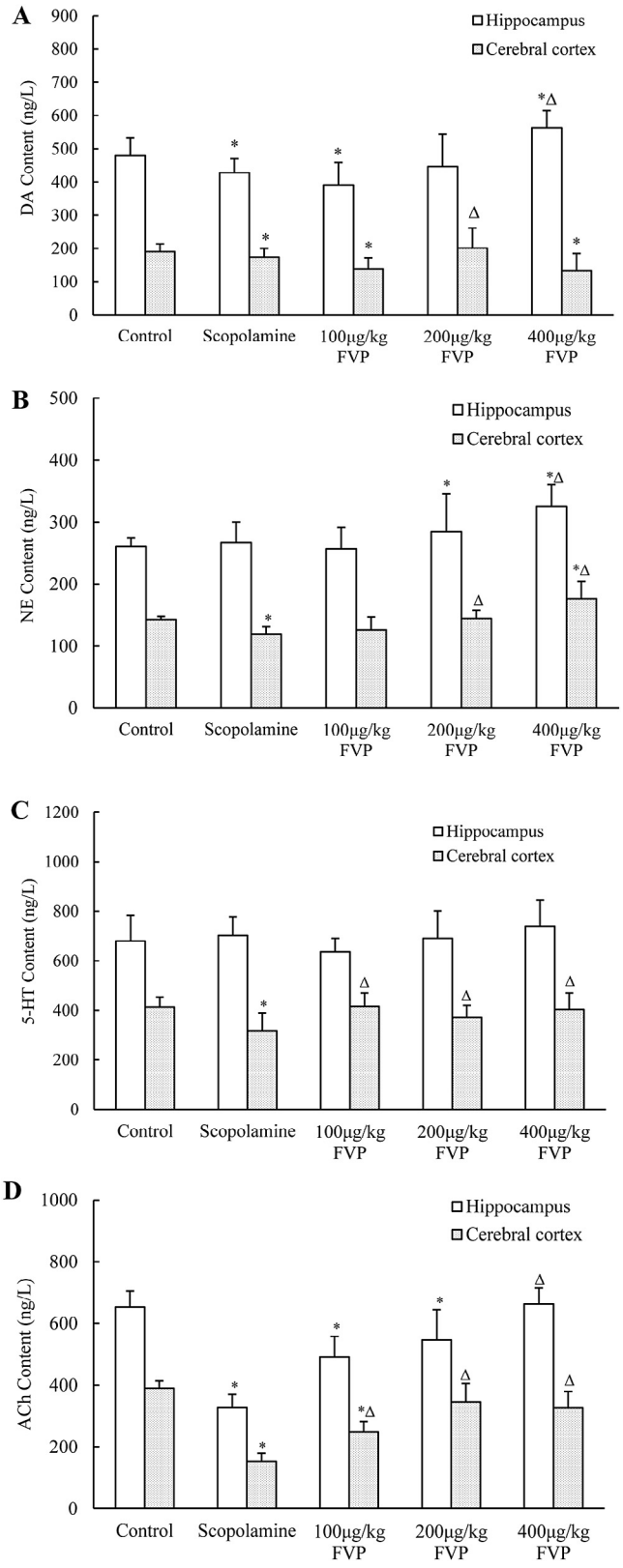


Fig. 4 – Contents of (A) DA, (B) NE, (C) 5-HT and (D) ACh in hippocampus and cerebral cortex of rats in different groups. DA: Dopamine, NE: Norepinephrine, 5-HT: 5-hydroxytryptamine, and ACh: Acetylcholine. *P < 0.05 vs control and ΔP < 0.05 vs scopolamine.

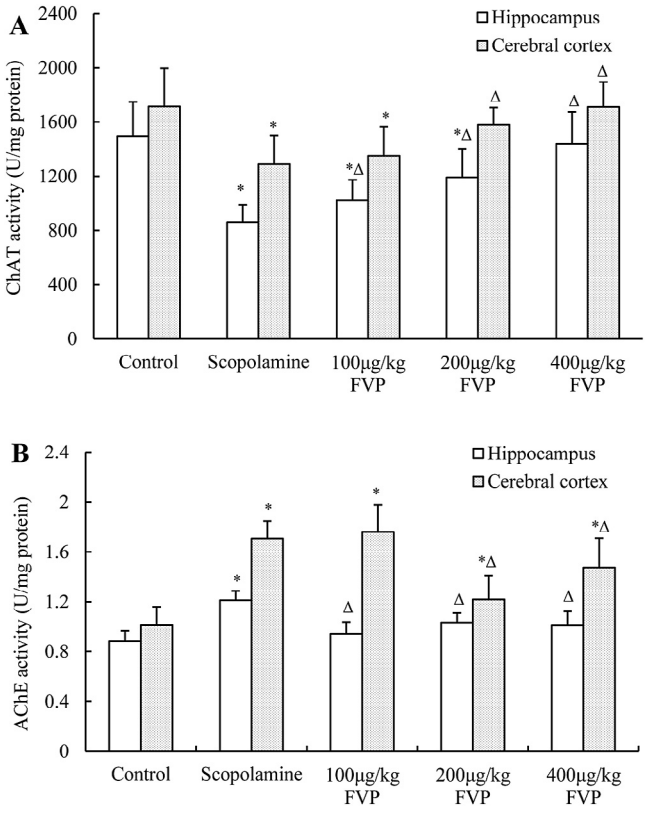


Fig. 5 – The ChAT (A) and AChE (B) activities in hippocampus and cerebral cortex of rats in different groups. ChAT: Choline acetyltransferase, AChE: Acetylcholinesterase. *P < 0.05 vs control and ΔP < 0.05 vs scopolamine.

from the brain extracellular fluid in the central nervous system. Some drugs have been proven to exert their neuroprotective effects by passing through the BBB. But it is believed that only some small molecules can cross the BBB via lipid-mediation. If the molecular weight (MW) of the drug is >400 Da, the likelihood of the drug crossing the BBB via lipid-mediation is low (Pardridge, 2009). The MWs of FVP-1 and FVP-2 were identified as 28 kDa and 268 kDa, respectively in our previous study (Yang et al., 2012). Therefore, it was difficult for FVP to pass through the BBB, and there is no evidence that polysaccharides could cross the BBB. Otherwise, polysaccharides have been proven to exert neuroprotective effects indirectly by enhancing antioxidative effects or the immune system's effectiveness (Gao et al., 2012). Therefore, to further elucidate the mechanism of FVP in improving learning and memory, the kinetics of FVP metabolism *in vivo*, whether the metabolites of FVP could pass through BBB, and the changes in hippocampus based on genome and proteomics will be studied in future works.

4. Conclusion

F. velutipes polysaccharides (FVP) were prepared and proven to improve spatial search behaviour of rats in MWM test. This excellent effect was mainly attributed to FVP elevating SOD and

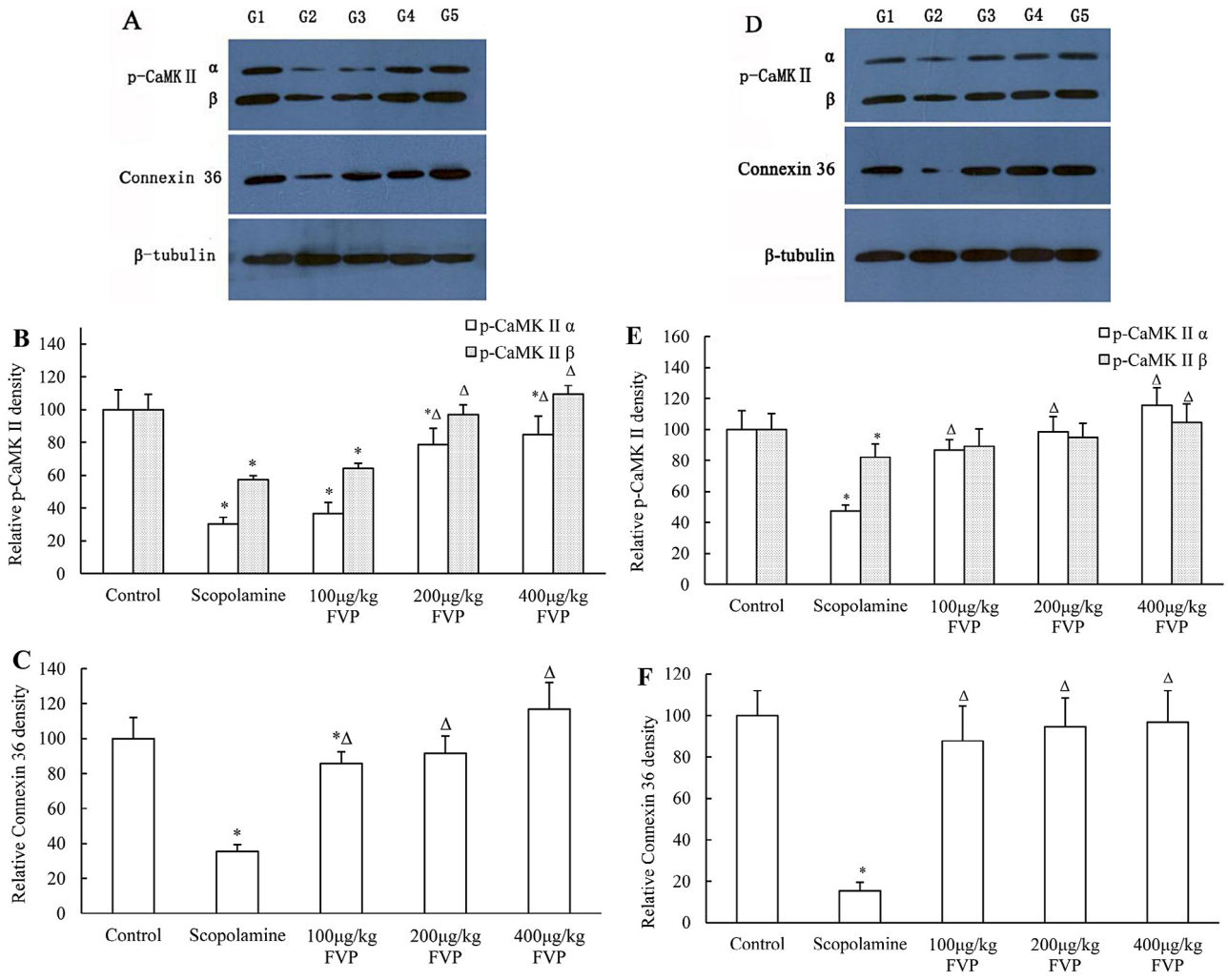


Fig. 6 – Expression of p-CaMK II and Connexin 36 in hippocampus and cerebral cortex of rats. (A) Western analysis of p-CaMK II and Connexin 36 in hippocampus; (B) the relative protein density of p-CaMK II in hippocampus; (C) the relative protein density of connexin 36 in hippocampus; (D) Western analysis of p-CaMK II and Connexin 36 in cerebral cortex; (E) the relative protein density of p-CaMK II in cerebral cortex; (F) The relative protein density of connexin 36 in cerebral cortex. G1: group 1 (Control); G2: group 2 (scopolamine-treated group); G3: group 3 (100 μg/kg·bw FVP); G4: group 4 (200 μg/kg·bw FVP); G5: group 5 (400 μg/kg·bw FVP). $^{*}P < 0.05$ vs control and $^{\Delta}P < 0.05$ vs scopolamine.

GSH-Px activities, restoring the level of ACh and modulating the ChAT and AChE activities in the hippocampus and cerebral cortex of rats. Furthermore, the expression levels of connexin 36 and p-CaMK II were elevated by FVP treatment. The present results conclusively indicated that FVP achieved its learning and memory improving function through a potential regulatory pathway: FVP elevated the expression of CaMK II and connexin 36, and then regulated the activities of ChAT and AChE to normalize the level of ACh. All these findings indicated that FVP could be used as a potential novel component in the development of functional foods to improve neuroprotective effect.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work is financially supported by the foundation of National Natural Science Foundation of China (No.31272220) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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